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STUDIES ON THE BIOCHEMISTRY AND CHEMO- THERAPY OF TUBERCULOSIS.*

I. THE PERMEABILITY OF TUBERCLES FOR IODIN COM- POUNDS AND PROTEINS.

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GENERAL CONSIDERATIONS.

The principles of chemotherapy, as laid down by Ehrlich, are of so fundamental a character that there is no limit to their application in infectious diseases, and possibly in other conditions, notably cancer. With the spirilloses and trypanosome infections, in which most of the work has so far been done, the conditions are favorable for the meeting of the drug and the germ, since with most forms of these diseases the germ lives chiefly or entirely in the circulating fluids. It is noteworthy that the only disease in which "Therapia magna sterilisans" has been practiced successfully on an empirical basis is also a blood infection, malaria. The consideration of tuberculosis from the standpoint of chemotherapy brings in distinctly new problems owing to the fact that the bacteria are, in large part, located at points specifically removed from the circulation by proliferating tissues. The avascularity of the tubercle must of necessity have a large influence on the meeting of the drug and the germ, and this condition has perhaps been responsible for the lack of success of the innumerable empirical attempts at chemotherapy which have been made with the disease in the past. Avascularity of an infected tissue may, perhaps, make for either assistance or hindrance in chemotherapy, for we can imagine that the drug might accumulate in the avascular area, just as, for instance, calcium salts do, or, entering avascular and vascular tissues alike, it might remain longer where there is no circulation. Absence of living cells may also make a difference in that certain drugs may be either destroyed or activated by living cells, and hence have either

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a greater or a less effect in the necrotic portions of the tubercle than elsewhere in the body. These and other points present themselves, and to attack the problem of tuberculosis chemotherapy it would seem to be necessary to learn first to just what extent different classes of chemical substances enter tubercles, both early and advanced, how much they tend to accumulate specifically in the tissues, and how long they remain there. For a chemical which is to destroy the tubercle bacillus, it would seem, should be one that will enter readily into the avascular tuberculous lesions, and, if possible, enter or accumulate in such tissues more than in normal tissues.

The problem is further complicated by the chemical composition of the tubercle bacillus itself, with its large proportion of resistant fatty and waxy material, which must, it would seem, make its permeation and destruction a very different matter from the attack upon the naked and delicate trypanosomes, spirillae, and spirochaetes. Hence the permeability of the tubercle bacillus for chemicals of different classes becomes a fundamental question in connection with the main problem. In the investigation of the subject the fatty matter of the tubercle bacillus, while perhaps an obstacle to chemotherapy, makes attack of the problem appear somewhat easier, since the permeability of the bacteria must be largely determined by this substance which can be extracted from them in large amounts and rendered available for experimental work *in vitro*, without, at the beginning, calling for the extensive animal experimentation which is essential in the study of the chemotherapy of protozoan infections. The influence of the fatty constituents of the cells upon the permeability of tissue cells to drugs and dyes has already been extensively investigated, and we have, therefore, many clues for investigation of the permeability of *B. tuberculosis*.

In planning a systematic investigation on the chemotherapy of tuberculosis, therefore, it seemed desirable first to determine the entrance of various classes of substances into the tubercle and into the bacteria, since the effective tuberculocide must be, theoretically, one which enters freely and, if possible, selectively into the avascular tubercle, and with like facility passes through the fatty

sheath of the bacillus. We have found it possible to attack directly some of the problems involved, while others have called for preliminary studies of certain fundamental questions. Some of the work has advanced sufficiently to warrant a preliminary report, which should be introduced by stating that all the work reported in this and in subsequent articles has been done through the co-operation of several persons, each of whom has helped in various stages so that it is difficult to credit any particular step to any one or two persons. Those engaged in various aspects of the work here reported are Dr. Lydia DeWitt, Dr. H. J. Corper, Dr. G. L. Kite, Miss Hope Sherman, Mr. G. C. Lake, and ourselves.

ENTRANCE OF SUBSTANCES INTO TUBERCLES AND OTHER LESIONS.

HISTORICAL.

The only study we can find directly concerning itself with this topic is that of Oswald Loeb and Michaud.¹ A study of the distribution of iodin in normal animals had been made previously by Loeb,² who found that when injected in the form of KI, the brain, spinal cord, bone marrow, and fat tissue were usually free from iodin, the muscle contained very little, and then, in increasing amounts, the liver, lymph glands, kidneys, salivary glands, lungs, blood, and, of course highest of all, the thyroid. When compounds of iodin which are soluble in fat were injected (iodoform, ethyl iodid) it was found in the brain, spinal cord, and fat tissue. After ethyl iodid injection he found the iodin especially in the lungs, where it is excreted, while more iodin is found in the kidneys and salivary glands after KI injection, for the same reason.

When these same iodin compounds were injected into tuberculous rabbits and guinea-pigs, Loeb and Michaud found that regularly the tuberculous tissues took up a disproportionately large amount of iodin. Thus, four rabbits inoculated in one eye with tuberculosis showed from one and one-half to two and three-fourths times as much iodin in the tuberculous eyes as in the normal eyes, and tuberculous lungs were found to contain increasing amounts of iodin in proportion to the amount of tuberculous tissue they contained. Caseous lymph glands of guinea-pigs contained more iodin than any of the normal organs.

This important investigation has only recently begun to receive the attention it has deserved, and has as yet been neither confirmed nor extended so far as tuberculous lesions are concerned, although in view of the fact that these weighty conclusions rest upon a series of four rabbits and four guinea-pigs, and that the relatively inaccurate method of Baumann was used for the iodin determination, amplification is certainly required before entire acceptance is warranted.

Collateral support is given by two observations on cancer. Van den Velden³ reported the case of a man who died of gall duct cancer, with secondaries in the liver

¹ *Biochem. Ztschr.*, 1907, 3, p. 301.

² *Arch. exp. Path. u. Pharm.*, 1907, 56, p. 321.

³ *Biochem. Ztschr.*, 1908, 9, p. 54.

and pancreas, five and one-half hours after a subcutaneous injection of 3.0 gm. of NaI. Analysis showed iodin in abdominal, pleural, and pericardial fluids, and in two large secondary growths in the liver and pancreas, but none in the normal liver and pancreas tissue, despite the relatively avascular nature of the tumors. The absence of iodin in the normal tissues under these conditions is difficult to understand, and, in the light of our observations on animals, incredible. Takemura,¹ who found that iodin distributes itself in normal rats and mice much as Loeb found in guinea-pigs, (noting an especially high content in the skin), observed that in mice with cancer there is nearly as much iodin in the tumor tissue after injection of KI as in the tissues which normally contain the greatest amount of iodin; in sarcoma in rats the iodin in the tumor tissue was intermediate between iodin-rich and iodin-poor tissues. Our own experiments, as given below, indicate that these results may depend upon the amount of necrosis in the carcinomas and sarcomas. More recently Loeb² has reported the finding of a larger proportion of iodin in the enlarged glands removed by operation from a syphilitic (0.28–0.53 mg. per gm.) than in the blood of the same patient (0.082–0.088 mg.) 20 hours after the last dose of iodides.

In this connection might be mentioned the solitary observation of Loeb that the pus in a turpentine abscess in a rabbit injected with KI contained a larger proportion of iodin than the blood itself. Also, the now classical observation of Bondi and Jacoby³ that injection of rabbits causes more of the injected salicylic acid to localize in joints, even when there is no arthritis; and the earlier observation by Fillipi and Nesti⁴ that after aspirin has been given by mouth to persons with arthritis, the synovial fluid contains more salicylic acid than the urine. Other related observations are the following:

Blumenthal⁵ observed that the addition of iodin to the atoxyl molecule causes it to enter into inoculable sarcomas of dogs and rats with special avidity, although even in the uniodized state the atoxyl is found more abundantly in these tissues than in the normal tissues. The effect of the atoxyl is to cause an increased rate of growth in the tumors of these animals.

Kapsenberg⁶ states that an extract of tubercle bacilli made with water in the presence of chloroform, has a decided affinity for iodin, and that the resulting compound is specifically bactericidal for tubercle bacilli, but the data presented in this article are not sufficient to carry conviction.

Morel and Dalous⁷ injected tuberculous guinea-pigs with anthrax cultures and found that the anthrax bacilli do not enter the larger tubercles; but in small tubercles consisting only of a giant cell and a single row of epithelioid cells, the protoplasm of each of these may contain anthrax bacilli.

EXPERIMENTAL.

We have undertaken to repeat the experiments of Loeb and Michaud, and to amplify them. Our plan of procedure was as follows: Guinea-pigs, the largest obtainable, were injected subcutaneously with human tubercle bacilli (0.01 mg. usually), and the animals were selected, as far as possible, when they had the maximum enlargement of the regional lymph glands before ulceration led to evacuation of their contents. In

¹ *Ztschr. physiol. Chem.*, 1911, 72, p. 78.

² *Arch. exp. Path. u. Pharm.*, 1912, 69, p. 108.

³ *Hofmeister's Beitr.*, 1906, 7, p. 514.

⁴ *Allg. med. Zentralztg.*, 1902, 71, p. 613.

⁵ *Deutsch. med. Wchnschr.*, 1910, 36, p. 2275.

⁶ *Berl. klin. Wchnschr.*, 1912, 49, p. 879.

⁷ *Compt. rend. Soc. de Biol.*, 1907, 62, p. 74.

order to secure local lesions which could be compared with corresponding normal tissues, several large males were inoculated in one testicle, but this did not give useful results, for invariably the other testicle developed extensive tuberculosis. Equally unsuccessful was the result of direct intra-hepatic inoculations, which caused only an extensive local miliary tuberculosis which rapidly became generalized. Good results were obtained by inoculating human tubercle bacilli into the vitreous of one eye, and then, just before the eyeball was ready to rupture, injecting the iodin compound and analyzing separately the normal and the tuberculous eye.

In order to learn whether the entrance of iodin compounds into tubercles depends upon some peculiarity of the tubercles themselves, or whether it is common to necrotic areas and exudates in general, a series of experiments was performed as follows: Necrosis of the entire left kidney was produced in rabbits by aseptic ligation of the artery, vein, and ureter. This is followed by a severe engorgement of the organ from the collateral circulation through the capsule, which results in stasis and total necrosis of everything but the capsule. Necrosis of muscle was produced by injecting 2 c.c. of 50 per cent formalin (equal to 20 per cent formaldehyde) into the muscle of one thigh; this also produces a severe local subcutaneous edema from which sufficient fluid could usually be expressed to permit of analysis. Exudates were produced by injecting into the left pleural cavity one to two gms. of aleuronat suspended in 5-10 c.c. water, sometimes with one c.c. of turpentine or a loopful of solid *Staphylococcus pyogenes aureus* culture added to produce more violent reactions. To secure an inert colloid mass to compare with the dead tissues, subcutaneous injections were made of sterile five per cent agar jelly, 8-20 c.c. being injected at 50° C. by means of a powerful syringe such as is used for cosmetic work with paraffin. Analysis of this agar showed it to contain a negligible amount of iodin, about 0.001 mg. per 20 c.c. in the amounts used. Several or all of these different procedures were carried out in the same animal in most instances, thus permitting a comparison of the iodin determinations in several different lesions as well as with the normal tissues of the same animal.

The injections were made subcutaneously with the following iodin compounds: Potassium iodid was used in five per cent solution, one c.c. of this solution (.050 gm. KI) generally being given per 100 gms. of animal weight as the standard dose. Iodoform was used in 10 per cent emulsion in olive oil, in doses of about one c.c. per 100 gms. animal weight. This was found to be more toxic than the other iodin compounds, especially for pregnant animals in which abortions usually resulted. Iodipin, 25 per cent iodin, was given in doses of about 0.5 c.c. per 100 gms. Ethyl iodid (Merck) was given in doses of about one c.c. per kilo. After the designated time had elapsed, the animals were bled from the carotids as thoroughly as possible, and in removing the tissues for analysis care was taken not to have errors arise from contamination with fluid from the site of the injection, which was always located as far as possible from the tissues that were to be examined. The tissues were finely ground in the fusion mixture, dried, and kept until ready for analysis. The large excess of alkali present in the fusion mixture seems, in view of the excellent results obtained in the subsequent analyses, to have been adequate to prevent any loss of volatile iodin compounds, even of the highly volatile ethyl iodid.

At first we made our analyses by the method devised by Hunter,¹ which possesses very evident advantages over the Baumann method, in that exact titration is used instead of the colorimetric method with its large subjective element, and especially

¹ *Jour. Biol. Chem.*, 1910, 7, p. 321.

in that it involves estimation of six times the amount of iodin originally present, which enormously reduces the error in determining iodin in small fractions of a milligram, as is necessary in this work. We soon found, however, that while we had no trouble in obtaining accurate results in our trial analysis of thyroid tissue or of test mixtures, yet during actual series of analyses there frequently occurred serious errors, sometimes total loss and sometimes large excess. In a number of instances these errors destroyed a large amount of work because they involved essential members of a series where duplicates were impossible. Investigation showed that there are several possible sources of error in this method. These have also been noted by others, especially by F. C. Kendall of New York, who published a preliminary report of an improved method¹ and who very kindly furnished us with a detailed account of his observations before their publication. Not securing altogether satisfactory results even with this method, a systematic investigation of the sources of error and the best means of correcting them was made by one of us (Hedenburg) and a method was at last devised which has been found altogether reliable and which possesses the aforementioned advantages of Hunter's method. This method will soon be described in detail in another publication.

The main results of our experiments are given in Tables 1 and 2, in which the figures represent milligrams of iodin per gram of fresh weight of tissue or fluid. Where more than one figure is given, without explanation, the results of duplicate or triplicate analysis are concerned, and these indicate well the limits of accuracy of the methods used. A dash indicates that no analysis was made. A question mark indicates that there is doubt as to the accuracy of the result obtained.

DISCUSSION OF RESULTS.

Examination of Tables 1 and 2 discloses the following facts: The methods of analysis used are sufficiently delicate and accurate nearly always to give reliable results, even with the small fractions of milligrams of iodin present in many of the samples analyzed. Occasionally, a result was obtained which was obviously entirely incorrect, and this is indicated in the table by a question mark. Such errors were observed chiefly when the original Hunter method was used, rarely with the newly devised modification of Hedenburg.

The relative amount of iodin found in the various tissues and organs seemed to show some variation with the form in which the iodin was compounded when injected, whether water-soluble KI or lipoid-soluble CHI₃, iodipin or ethyl iodid. But the results are not altogether in harmony with the observations of Loeb and others on the organotropic character of the partition of different forms of iodin compounds. We do not attempt to interpret these disagreements, as our problem lies elsewhere, but give the figures without

¹ Proc. Soc. Exp. Biol. and Med., 1910, 8, p. 120; complete report in Jour. Amer. Chem. Soc., 1912, 34, p. 894.

further comment. In a few experiments, extraction was performed with absolute ether, and the tissues were prepared for extraction by grinding to powder with anhydrous sodium sulfate. The ether extract was then shaken out with water to remove any traces of iodides and evaporated to dryness after mixing with fusion mixture. It will be noted that a little iodin was found in the brain and fat tissues even when it was given in the form of KI. No differences were observed between the results with rabbits and with guinea-pigs.

Potassium iodid given subcutaneously is eliminated rapidly, so that there is very little left in the blood or tissues even 12 hours later. With iodoform and iodipin very irregular results were obtained, which perhaps depend upon local conditions modifying absorption. Thus, in an experiment with iodipin, none at all could be found in the blood or organs of one animal (19, Table 1), while other animals with somewhat larger doses showed considerable amounts. Also, of three guinea-pigs given iodoform in much the same way, the amount of iodin in the blood was respectively 0.205, 0.006, and 0.120 mg. per cubic centimeter of blood. We have not attempted to determine how large a proportion of the iodin found in the blood and tissues is in the form injected and how much is inorganic iodids.

The blood practically always contains more iodin, no matter in what compound it is given, than any tissue or organ, whether normal or otherwise. Occasionally during active excretion the kidney will be found to contain more iodin than the blood, and generally it contains nearly as large a proportion weight for weight, as the blood of the same animal. The relation is well shown in the following parallel columns from a series of analyses under various conditions of dosage, time, iodin compound, and kind of animal used (guinea-pigs and rabbits).

On account of its function of excreting iodin the kidney cannot be compared with other tissues. Of the chief viscera, the liver seems to be perhaps the most stable as to the proportion of iodin, but as a rule it contains much less iodin than the blood, generally about one-third as much per gram. Thus of 29 analyses under the varied conditions of these experiments, in three the liver contained approximately the same amount of iodin as the blood, in four it contained

TABLE I.
IODIN IN NORMAL AND TUBERCULOUS TISSUES.

Animal	Dosage	Interval between Injection and Death	Blood	Liver	Spleen	Kidneys	Lungs	Tuber-culous Glands	Miscellaneous	Autopsy Findings and Remarks
1. (Guinea-pig) 770 gms . . .	o.385 gm. KI	6 hrs.	?	o.064 (left) o.072 (right)	o.094	o.115	?	o.092	Testes, o.110 Urine, 19.0 mg.	KI injected intravenously (in all other experiments subcutaneously). Lymph glands hard, large, full, some caseous; analyzed together, wt. 5.9 gms. Spleen ridged with tubercles, many smaller ones in liver, a few in lungs.
2. " 600 gms . . .	o.300 gm. KI	48, 24, and 6 hrs.	o.179 o.218	?	o.074	o.167	o.151	o.160	Testes, o.121	Glands large and caseous in inguinal region, hard and large in mediastinal, pelvic, and pancreatic; analyzed together, wt. 6 gms. Many 2-3 mm. tubercles in spleen, smaller ones in liver.
3. " 480 gms . . .	o.250 gm. KI	48, 24, and 6 hrs.	o.337	o.108 (left) o.110 (right)	o.071	o.034	o.147	o.203	Inguinal glands only slightly enlarged and caseous, pancreatic and mediastinal enlarged and hard; analyzed together, wt. 7.6 gms. Extensive miliary tubercles in liver and lungs, larger in spleen. Bled incompletely.
4. " 400 gms . . .	o.200 gm. KI	72, 48, 24, and 6 hrs.	o.388 o.433	o.365 (left) o.333 (right)	lost	o.518	o.348	o.295 o.481 pus	Inguinal glands contained, softened material analyzed separately (o.9 gm.) from the rest of the enlarged glands (5.5 gms.). Many 1-2 mm. tubercles in spleen, a few in lungs and liver.
5. " 720 gms . . .	o.350 gm. KI	48, 24, and 6 hrs.	o.680 o.430	o.567 o.532 o.535	o.108	1.168	o.213	o.285 o.790 wall o.369 pus	Large caseous axillary glands containing 3.3 gms. softened material analyzed separately from the wall of the cavity, wt. 2.2 gms.
6. " 370 gms . . .	o.2 gm. KI	6 and 3 hrs.	o.558	o.101	o.290	o.369	Eye, normal	Large caseous cervical glands.
7. (Rabbit) 1,750 gms . . .	o.9 gm. KI	4 hrs.	o.479 o.472	o.132 (left) o.153 (right)	tuberculous wall o.381 o.020 o.032	Extensive tuberculosi of choroid; this eye weighing 4.5 gms. and containing 1.7 mg. iodin; normal eye wt. 2.2 gms. iodin o.48 mg. No tuberculosis elsewhere.
8. " 1,500 gms . . .	o.750 gm. KI	6 hrs.	o.171 o.168	o.025 o.035 (right)	o.151	o.031	Brain Fat o.182 tuberculous	Moderate tuberculosis of choroid, eye weighing 3.6 gms. and containing 0.54 mg. iodin; normal eye wt. 2.7 gms.; iodin o.492 mg. No tuberculosis elsewhere.
9. " 1,800 gms . . .	o.9 gm. KI	8 hrs.	o.194 o.179	o.022 (left) o.020 (right)	o.003	Eye, normal tuberculous o.078	Tuberculous eye weighed 4.0 gms. iodin o.67 mg.; normal eye wt. 3.2 gms. iodin o.25 mg. No tubercles in other organs.
10. " 1,500 gms . . .	o.750 gm. KI	12 hrs.	o.006 o.011	o.006 o.011	o. o	o	o	o	Brain Fat o.015 No iodin in either eye	Normal eye wt. 2.8 gms., tuberculous wt. 4.8 gms. Not sufficient iodin to be detected in any of the organs.

11. (Guinea-pig) 550 gms.	48 hrs.	o.178 (left) o.071 (right)	o.100	o.111	o.100	o.152
12. " 680 gms.	72 and 24 hrs.	o.093 o.093	o.079	o.118	o.087	o.126	Muscle, o.064	Pregnant, died without being bled.
13. " 800 gms.	6, 5, 3 days	o.205	o.060 o.033 tubercles	o.013	?	o.090	Fat o.013	Large caseous inguinal glands, 3.0 gms. Many miliary tubercles in spleen, a few in liver and lungs. Bled fairly well. Large partly caseous inguinal and hard, pancreatic and mesastinal glands analyzed together, wt. 6.1 gms. Liver very fatty.
14. " 600 gms.	5 and 3 days	o.006	o.002 o.002	o	o	o.003	Testes o.007 capsule Fat o.013 pus ether extract	Spleen full of tubercles. Not well bled. Some large caseous areas isolated from rest of liver tissue and analyzed separately. (o.8 gm.). Liver fatty. Spleen full of tubercles; both testicles found full of small tubercles. Few tubercles in liver. Large abscess in left axilla. (pseudotuberculosis?) the pus of which was extracted with ether and extraction and residue analyzed separately.
15. (Rabbit) 1,600 gms.	5 c.c. to per cent CHI ₃	5 and 3 days	o.120 o.121	o.025 o.002 ether extract	Eye, normal o.038 tuberculous	Inoculated in right eye, which was nearly ready to rupture, wt. 4.8 gms. Normal eye wt. 1.28 mg.; normal eye wt. 4.0 gms. Iodin r.15 mg. No tuberculosis elsewhere.
16. " 2,000 gms.	10 c.c. to per cent CHI ₃	40 hrs.	o.217	o.172 (left) o.180 (right)	o.126	Died from toxoform, and not bled, therefore all organs full of blood. Normal eye, wt. 3.0 gms., total iodin 0.279 mg.; tuberculous eye wt. 4.8 gms.; iodin o.578 mg. No tuberculous elsewhere.
17. (Guinea-pig) 550 gms.	3 c.c. iodipin	72, 36, and 12 hrs.	o.019 o.031	o.080 o.053 (left) (right)	o.195	o.032 o.111	Brain o.069 Fat o.219	Ingoinal glands very large and caseous, some caseous in pancreatic and mesastinal glands; analyzed together, wt. 11.5 gms. Spleen almost entirely tuberculous, slight in liver and lungs. Thoroughly bled.
18. " 600 gms.	3 c.c. iodipin	72 and 24 hrs.	o.082	o.093 (left) o.091 (right)	o.016	o.086 o.044	Glands showed only slight caseation; wt. together 4.9 gms. Not much tuberculosis in other organs. Bleed thoroughly.
19. " 600 gms.	4, 3 and 1 day	o	o	o	Testes o.021	Inoculated in testicles. Extensive tuberculosis in all organs, especially lungs. No iodin could be found anywhere. Tuberculous eye wt. 5.2 gms., iodin 0.933 mg.; normal eye 2.0 gms., iodin 0.075 mg. No other tuberculosis.
20. (Rabbit) 1,750 gms.	4.5 and 2.25 c.c. iodipin	3 and 1 day	o	o.009 (left) o.008 (right)	Brain o.0002 Fat o.045	Eye, normal o.028 tuberculous
21. " 1,700 gms.	2.5 c.c. ethyl iodid	6 hrs.	o.215 o.207	o.042 (left) o.036 (right)	o.080	Normal eye wt. 2.2 gms., iodin o.062 mg.; tuberculous eye 5.8 gms., iodin o.86 mg. No tuberculosis elsewhere.
							Brain o.003 Fat o.148	

TABLE 2.
IODIN IN NECROTIC TISSUES AND EXUDATES.

Animal	Dosage	Interval between Injection and Death	Blood	Liver	Spleen	Kidneys	Lungs	Muscle	Exudate	Miscellaneous	Autopsy Findings and Remarks
1. (Guinea-pig) 370 gms.	0.38 gm. KI	6 hrs.	0.488	0.101	0.139	0.248	0.312	0.205	0.087	Bile o.221	o.314 8 c.c. agar injected subcutaneously 60 hrs. before bleeding, which was incomplete. Agar found well encapsulated; much edema in surrounding tissue, which was analyzed separately. Duplicate of No. 1, but not bled until 6 days after agar injection.
2. " 450 gms.	0.45 gm. KI	6 hrs.	?	0.077 (left)	0.107	0.112	0.100	Bile Glands o.218	o.310 Duplicate of No. 1 and 2, bled after 9 days.
3. " 720 gms..	0.35 gm. KI	48, 24, and 6 hrs.	0.680	0.108	0.168	0.213	0.280	Capsule about agar o.390	Duplicate of Nos. 1 and 2, died without being bled.
4. (Rabbit) 3450 gms...	1.5 gm. KI	18 and 1 hr.	0.812	0.365 (left)	0.485 (left)	0.679 (left)	0.429	0.435	0.139	Abcess pus o.303	Left kidney ligated 3 days and agar injected 9 days before death, and also agar and staphylococci to produce subcutaneous abscess. Died without being bled. Advanced pregnancy. Liver very cirrhotic. Left kidney entirely necrotic, wt. 23.2 gms., contained 15.7 mg. iodin; right kidney wt. 11.4 gms.; iodin 4.8 mg. Spleen agar well encapsulated; infected agar in abscess cavity.
5. " 2,140 gms...	1.06 gm. KI	48, 24, and 6 hrs.	0.524 o.536	0.187 (left)	0.317	0.487 (left)	0.500 (right)	0.095	Hematoma in muscle o.352	Left kidney ligated 5 days before death. Bleed only fairly thoroughly. Left kidney entirely necrotic, wt. 11.2 gms; iodin 5.4 mg.; right kidney 5.2 gms.; iodin 2.6 mg. Hematoma in pease muscle analyzed separately.

6. (Rabbit) 2,470 gms... K1	1.24 gm. 25 and 6 hrs.	0.425 0.114 (left) 0.131 (right)	0.178 0.539 (left) 0.389 (right)	0.061? 0.147 (left) 0.147 (right)	0.159	Left kidney ligated 5 days before bleeding; 3 days later injected aeuronat into left pleural cavity. Bleed well. Much turbid exudate and fibrin in pleura, analyzed together, wt. 11 gms. Left lung collapsed and congested. Left kidney necrotic, wt. 14.2 gms.; iodin 6.7 mg.; right wt. 9 gms.; iodin 3.5 mg. Duplicate of No. 6. Bled poorly. Resembled No. 6 at autopsy. Left kidney 9.7 gms.; iodin 2.6 mg.; right, 6.6 gms.; iodin 2.1 mg.
7. " 1,500 gms... K1	0.75 gm. 24 and 6 hrs.	0.391 0.681 (left) 0.804 (right)	0.221 0.267 (left) 0.319 (right)	0.184	0.343	Duplicate of No. 6. Bled poorly. Resembled No. 6 at autopsy. Left kidney 9.7 gms.; iodin 2.6 mg.; right, 6.6 gms.; iodin 2.1 mg.
8. " 2,000 gms... K1	1.0 gm. 22 and 6 hrs.	0.393 0.306 (left) 0.105 (right)	0.123 0.403 (left) 0.530 (right)	0.030 normal 0.361 pleura 0.300 necrotic	0.310 edema 0.401 fibrin in pleura	Kidney ligated 12 days; formalin injected into muscle 4 days and aeuronat - stabphylococcus suspension into left pleura 2 days before bleeding thoroughly. Heavy fibrinous purulent pleuritis and pericarditis; separated fibrin from fluid and analyzed separately. Left lung collapsed. Left kidney necrotic, wt. 30.5 gms.; iodin 12.3 mg.; right kidney 8.2 gms.; iodin 4.35 mg. Muscle necrotic where formalin injected; much subcutaneous edema fluid, analyzed separately. Focal necrosis in liver.
9. (Guinea-pig) 620 gms. K1	0.31 gm. 3 days, 29 hrs.	0.0 0.007	0.010	? 0.011	0.020	Fat 0.030 Agar injected 9 days before bleeding. Necrosis of skin followed first K1 injection.
10. " 600 gms. K1	0.3 gm. 4 and 2 days	0.005 0.006	0.004 0.006	? 0.016	0.022	Brain 0.030 Fat 0.030

TABLE 2—Continued.

Animal	Dosage	Interval between Injection and Death	Blood	Liver	Spleen	Kidneys	Lungs	Agar	Muscle	Exudate	Miscellaneous	Autopsy Findings and Remarks
II. (Rabbit) 1,400 gms...	0.35 gm. KI	36 and 12 hrs.	0.016 0.023	0.008 0.007	?	0.00 (left) 0.01 (right)	0.016	0.916?	0.004 normal 0.07 necrotic	0.020 pleura 0.014 edema	Kidney ligated 8 days, aleuronat injected subcutaneously and formalin injected into muscle 3 days, agar injected 6 days before bleeding thoroughly. Aleuronat found encapsulated. Muscle necrotic and much subcutaneous edema where formalin was injected; fluid analyzed separately. Left kidney wt. 11.9 gms., iodin 0.24 mg.; right 6.9 gms., iodin 0.09 mg.
I2.	" 1,800 gms...	5 c.c. 10 per cent CHI ₂	3 days	0.014	0.001 (left) right lost	0.012	0.001 normal 0.091 necrotic	Brain, ether extract Residue 0.025
I3.	" 1,700 gms...	5 c.c. 10 per cent CHI ₂	6 days	0.072 0.072	0.032	?	0.009 (left) 0.009 (right)	0.088 0.071 (right)	0.077	0.013 normal 0.100 necrotic	0.078 Bile Fat	Brain, ether extract Residue 0.014 0.076
I4.	" 1,500 gms...	3 c.c. 25 per cent iodipin	6 days	0.006 0.007	0.002 0.002	0	0.001 (left) 0.002 (right)	0.001	0.010	0.0 normal 0.001 necrotic	0.0035 Brain Fat	Brain, ether extract Residue 0.005

15. (Rabbit) 1,750 gms...	1.75 c.c. ethyl iodid	6 hrs.	0.168 0.161	0.060 0.052	0.030	0.043	0.066 (left) 0.236 (right)	0.072	0.018 normal necrotic	0.105 aleu- ronat edema	Brain o.001 residue ether extract o	Duplicate of No. 11. Aleu- ronat injected into peri- toneum and removed as solid dry lump. Brain re- moved and ether extract analyzed. Left kidney wt. 11.1 gms., iodin o.8 mg.; right 5.1 gms., iodin 1.3 mg.
16.	" 2,000 gms...	2 c.c. ethyl iodid	6 hrs.	0.136 0.124	0.039 0.042 0.020 ether extract	0.019 (left) 0.120 (right)	0.024	0.012 normal o.066 solid	Brain, ether extract o	Brain, ether extract o	Duplicate of No. 15, except aleuronat and turpentine emulsion was injected into left pleura, causing much exudate and collapse of left lung and some solidification, fibrin of exudate analyzed separately from fluid. Liver showed exten- sive focal necrosis. Left kidney, wt. 14.8 iodin o.4 mg.; right 6.2 gms., iodin o.75 mg.
17.	" 2,500 gms...	2.5 c.c. ethyl iodid	12 hrs.	0.433	0.102 (left) 0.097 (right)	0.070	0.214 (right) 0.320 (left) lost	0.283	0.040 normal o.064 necrotic	0.336	Brain o.021	Duplicate of No. 16 but died when bleeding was started, and therefore incompletely bled. No focal necrosis of liver.

a very little more and this when there was but very little iodin present (three were iodipin and one KI experiments); while of the 22 in which the blood contained the most iodin, in 16 the excess was in a ratio between two and four to one.

TABLE 3.
RELATIVE IODIN CONTENT OF KIDNEYS AND BLOOD.

	Blood	Kidney	Injection	Time Elapsed after Injection
I.....	.488	.248	KI	6 hours
2.....	.680	1.168	KI	48, 24, and 6 hours
3.....	.812	.420	KI	18 and 1 hour
4.....	.524	.500	KI	48, 24, and 6 hours
5.....	.425	.389	KI	24 and 6 hours
6.....	.391	.319	KI	24 and 6 hours
7.....	.306	.530	KI	22 and 6 hours
8.....	.005	.000	KI	4 and 2 days
9.....	.019	.011	KI	36 and 12 hours
10.....	.093	.118	CHI ₃	72 and 24 hours
11.....	.072	.069	CHI ₃	6 days
12.....	.025	.195	iodipin	72, 36, and 12 hours
13.....	.060	.016	iodipin	72 and 24 hours
14.....	.130	.120	C ₂ H ₅ I	6 hours
15.....	.432	.214	C ₂ H ₅ I	12 hours
16.....	.168	.256	C ₂ H ₅ I	6 hours

The statement of Loeb that the left lobe of the liver regularly contains less iodin than the right we can corroborate in part only. In only two of 19 livers examined was there appreciably more iodin in the left, and in 10 there was definitely more in the right. In the remainder there was no difference above the limit of error of analysis. Generally, the ratio varies between 5:6 and 7:8. The two exceptional results were obtained with iodoform and iodipin.

As a rule, we found less iodin in the lungs than in the liver, but often the amount is about the same, and it is not uncommon to find more in the lungs, especially when only traces are left in the body. In four experiments when ethyl iodid had been given, we observed an excess of iodin in the lungs in two, and in the liver in two, which does not entirely corroborate Loeb, but our figures are too few to be significant.

The figures for the spleen vary greatly, perhaps because of the small quantity of material available for analysis; all in all it ranked about the same as in the liver. Herein we fail to corroborate Boruttau, who states that lymphatic tissues take on an excess of iodin, but corroborate Loeb (1912).

In all cases the muscle content runs far below that of all the

other tissues, except the brain, containing usually but one-half to one-third as much as the liver. Thus, the average of 12 analyses of liver and muscle from the same animals under varying conditions, showed 0.115 mg. iodin per gram liver and 0.041 mg. per gram muscle. In only two cases did the muscle have as much as half the amount present in the liver. The testicles seem to take on about as much iodin as the liver, and apparently the bile is an important avenue of escape of iodin from the blood.

The effects of pathological changes upon the tissues were very definite. Tuberculous lymph glands do, as Loeb first showed, take up in general relatively more iodin from the blood than do the liver, spleen, and lungs of the same animal. Thus, in nine of 11 experiments the tuberculous lymph glands contained more iodin than the liver, and in the best experiments with KI the amount approaches that in the blood. See Table 4.

TABLE 4.
IODIN IN CASEOUS GLANDS.

No. in Table 1	Blood	Liver	Caseous Glands	Injection	Time after Injection
I.....	?	.068	.092	KI	6 hours
2.....	.195	?	.160	KI	48, 24, and 6 hours
3.....	.337	.109	.203	KI	48, 24, and 6 hours
4.....	.408	.400	.481	KI	78, 42, 24, 6 hours
5.....	.550	.580	.790	KI	48, 24, and 6 hours
11.....100	.152	CHI ₃	48 hours
12.....	.093126	CHI ₃	72 and 24 hours
13.....	.205	.060	.013	CHI ₃	6, 5, and 3 days
14.....	.006	.002	.013	CHI ₃	5 and 3 days
17.....	.025	.065	.111	iodipin	72, 36, and 12 hours
18.....	.082	.092	.044	iodipin	72 and 24 hours
19.....	.0	.0	.0	iodipin	4, 3, and 1 day

It is especially noticeable that when the caseous material was abundant enough to permit of separation from the rest of the gland substance, it contained much more iodin than did the non-caseous portion of the glands, as seen in experiments Nos. 4, 5, and 14, where the figures are:

	4	5	14
Gland substance	0.295	0.285	0.007
Caseous contents	0.481	0.790	0.013

In only a few instances was there a noticeable deficit in iodin in the tuberculous tissues. In experiment No. 13 a small amount of necrotic liver tissue (0.9 gm.) seemed to contain less iodin than the

rest of the liver, but the amount of iodin involved is so small that the results are of doubtful reliability. The fact that here and in the glands in Nos. 13 and 18 the amount of iodin is lower in the caseous tissue than in the normal liver, may be ascribable to a relative chemotropism of the liver for iodoform and iodipin used in these experiments.

Tuberculous lesions in the eye show, as was also found by Loeb and Michaud in four experiments, an increased capacity for taking up iodin, as shown by the following summary from Table 1.

TABLE 5.
IODIN IN TUBERCULOUS AND NORMAL EYES.

NO. IN TABLE I	WEIGHT OF EYE		TOTAL IODIN		MG. IODIN PER GRAM		FORM OF IODIN INJECTED
	Normal	Tuber- culous	Normal	Tuber- culous	Normal	Tuber- culous	
7.....	2.2	4.5	0.48	1.7	0.220	0.381	KI, 4 hours
8.....	2.7	3.6	0.49	0.54	0.182	0.150	KI, 6 hours
9.....	3.2	4.0	0.25	0.67	0.078	0.166	KI, 8 hours
10.....	2.8	4.8	0	0	0	0	KI, 12 hours
15.....	4.0	4.8	0.15	1.38	0.038	0.267	Iodoform
16.....	3.0	4.8	0.28	0.58	0.093	0.117	Iodoform
20.....	2.0	5.2	0.015	0.033	0.0075	0.006	Iodipin
21.....	2.2	5.8	0.062	0.86	0.028	0.148	Ethyl iodid
Average.....	2.76	4.7	0.216	0.720	0.081	0.154	

Of these eight experiments, without exception the amount of iodin is greater in the tuberculous eye than in the normal eye, although in two (8 and 20) the proportion of iodin is slightly greater in the normal eye. Taken all together, there is over three times as much iodin in the tuberculous eyes, and nearly twice as large a proportion. The low figure for iodipin (No. 20) corresponds entirely with the proportion of iodipin in the liver of the same animal (0.008), and it is evident that after injections of iodoform and ethyl iodid the iodin readily enters the eyes, especially the tuberculous eyes, although whether as organic or inorganic compounds we have not ascertained.

That the entrance of iodin into tuberculous tissue is not characteristic of tuberculosis is established by the analyses of the tissues of animals in which necrosis and exudates were produced experimentally (Table 2). In 10 rabbits which had the left kidney rendered totally necrotic by ligation of all the blood vessels, there

is found to result a great increase in the size of the organ, from an average of 7.2 gms. to 15.3 gms., because of hemorrhage and edema. In spite of the avascularity of these kidneys, iodin permeates them rapidly, so that six hours after injection there is found to be, on the average, almost identically the same proportion of iodin in the avascular necrotic kidney and in the normal kidney, a proportion which, as pointed out previously, approximates that of the iodin content of the blood more closely than in any other organ. These facts are shown in Table 6, summarized from Table 2. Therefore, it seems evident that in a short time, the iodin in the blood will penetrate even so large an avascular area as an entire kidney, and reach practically the same concentration as in the blood itself.

TABLE 6.
IODIN IN NORMAL AND NECROTIC KIDNEYS.

NO. IN TABLE 2	GMS. WEIGHT OF KIDNEYS		TOTAL MG. IODIN IN KIDNEYS		MG. IODIN PER GM. OF		
	Necrotic	Normal	Necrotic	Normal	Blood	Necrotic Kidney	Normal Kidney
4.....	23.2	11.4	15.7	4.8	.812	.679	.420
5.....	11.2	5.2	5.4	2.6	.530	.487	.500
6.....	14.2	9.0	6.7	3.5	.425	.539	.389
7.....	9.7	6.6	2.6	2.1	.391	.267	.319
8.....	30.5	8.2	12.3	4.4	.305	.403	.530
IX.....	11.9	6.9	0.2	0.1	.019	.020	.011
X.....	12.8	6.9	12.6	4.7	.072	.099	.069
XI.....	12.1	5.1	10.8	1.3	.168	.066	.256
XII.....	14.8	6.2	0.4	0.8	.130	.026	.120
Total.....	140.4	65.5	66.7	24.3	2.852	2.586	2.614
Average.....	15.6	7.3	7.4	2.7	.317	.287	.290

Of all the tissues, however, the normal kidney alone seems to be so permeable for iodin that it comes to contain the same proportion as the blood, a fact which is presumably related to the functional activity of the organ. If we take another tissue which is not normally so permeable for iodin, such as the muscle, we find the interesting fact that necrotic areas in this tissue also tend to contain approximately as much iodin as the blood of the same animal, while the normal muscle tissue, in spite of its much greater blood supply, contains much less iodin. This fact is shown in Table 7.

In the above series the necrosis was produced by injection of strong formalin (except No. 5, in which trauma during operation

probably caused the injury). In removing the tissues at autopsy, the necrosis not being sharply circumscribed, more or less normal tissue was probably always included with the necrotic muscle; if only completely necrotic muscle had been present in these samples, it is probable that the proportion of iodin would have been still higher.

TABLE 7.
IODIN IN NORMAL AND NECROTIC MUSCLE.

No. in Table 2	Blood	Normal Muscle	Necrotic Muscle
I.....	.488	.087
4.....	.812	.139
5.....	.530	.095	.352
6.....	.425	.061
8.....	.305	.030	.300
II.....	.019	.004	.017
12.....001	.001
13.....	.072	.013	.100
14.....	.006	.000	.001
15.....	.165	.018	.052
16.....	.130	.012
17.....	.433	.040	.064
Average of analyses where all three figures were obtained.....	.219	.029	.126

The explanation of these results, it seems to us, must be as follows: The partial impermeability of living cells, which presumably differs in all organs and cells, is destroyed when the cell is killed. Therefore, the readily diffusible iodin compounds present in the blood and tissue fluids will diffuse into the necrosed tissue elements just as they would into any inert water-filled colloidal mass, with the resulting tendency, as shown by our figures, to approach osmotic equilibrium of iodin in blood and necrotic tissue. The large amount of iodin present in necrotic tissues, whether tuberculous or otherwise, is, therefore, dependent on purely physical conditions, i.e., the destruction of the semi-permeability of the cells. That it does not depend upon any chemical attraction, or even a specific physical "adsorption," is shown by the fact that if some time is allowed for the iodin to be excreted in part from the body after injection, it leaves the necrotic tissues, the blood and the normal tissues *pari passu*. Support is given to this interpretation by the results of implantation of agar into the subcutaneous tissue, followed by injection of iodin compounds at various intervals. The agar was introduced at a temperature of about 50° C.,

and solidified in a lump which soon became encapsulated, and after a time permeated by invading strands of granulation tissue. The results of analyses from several such experiments are given in Table 8.

TABLE 8.
IODIN IN AGAR.

No. in Table 2	Blood	Liver	Agar
1.....	.488	.101	.265
2.....	..	.089	.100
3.....	.680	.580	.280
4.....	.812	.500	.435
9.....	..	.007	.020
10.....	.005	.004	.022
12.....	..	.014	.012
13.....	.072	.032	.077
14.....	.006	.002	.010
15.....	.168	.056	.072
16.....	.013	.040	.024
17.....	.433	.100	.283

These experiments seem to show a marked permeability of agar for iodin. In experiment No. 4, for example, although the tissues were examined only one hour after injection of the iodid, yet even this quickly the avascular agar contained as much iodin as the liver, which in this case gives an abnormally high figure because the animal could not be bled. In eight of 12 experiments, the agar contains a larger proportion of iodin than the liver, in only one is it considerably less. It will be noticed, also, that in this series as in all the other experiments the form in which the iodin is introduced seems to make little difference in its distribution.

As is to be expected, inflammatory exudates are prone to approach the blood in iodin content. Table 9 shows no evidence of any selective tendency of iodin to enter the inflammatory exudate, however, there nearly always being somewhat less iodin in the exudate than in the blood. The presence of iodin in the exudate would seem in all cases to be dependent entirely on simple diffusion, as in the case of necrotic tissues and implanted agar.

The high iodin content of tuberculous eyes is presumably to be explained, therefore, as due in part to the inflammatory exudate present in these eyes, and probably in less degree, to the necrosis in the tuberculous tissues.

Similarly, compression atelectasis of the lung, produced by pleural exudates, with the resulting greater or less edema and

inflammatory exudate in the alveoli, is associated with a corresponding slight increase of iodin in the injured lung, as shown in Table 10.

TABLE 9.
IODIN IN EXUDATES.

No. in Table 2	Blood	Liver	Exudate	Character of Exudate
I.....	.488	.101	.221	Edema about agar
4.....	.812	.500	.503	Subcutaneous abscess
6.....	.425	.125	.159	Serofibrinous, pleural
7.....	.391	.720?	.343	"
8.....	.305	.100	.310	Edema, subcutaneous
			.361	Pleural fluid
			.461	" fibrin
II.....	.019	.007	.020	Aleuronat subcutaneously
			.014	Edema, subcutaneous
I3.....	.072	.032	.078	Serofibrinous pleuritis
I4.....	.006	.002	.0035	" "
I5.....	.165	.059	.0025	Edema, subcutaneous
			.130	" "
I6.....	.130	.040	.105	Aleuronat, intraperitoneal
			.090	Pleural fluid
I7.....	.433	.100	.067	" fibrin
			.336	Serofibrinous pleuritis

TABLE 10.
IODIN IN NORMAL AND COLLAPSED LUNGS.

No. in Table 2	Blood	Normal Lung	Collapsed Lung
6.....	.425	.147	.040?
8.....	.305	.189	.326
I3.....	.072	.071	.088
I6.....	.013	.020	.019
I7.....	.433	.230	.320

These observations would seem to explain entirely, and on a simple physical basis, the observation of Bondi and Jacoby, Fillipi and Nesti, and Loeb, that drugs tend to enter inflammatory exudates. They enter simply because there is present an exudate which offers no resistance to their permeation to establish an osmotic equilibrium with the blood, and not because there is a specific affinity between pathological tissues and blood. Therefore, we are led to the conclusion that *the supposed affinity of certain drugs for certain pathological tissues merely depends on a decreased impermeability of the diseased cells, or diffusion into inflammatory exudates present in the diseased area, or both.* If this is the case, we might expect a non-diffusible colloidal substance to be unable to penetrate avascular diseased areas which are highly permeable for crystalloids, and this was found to be true. Tubercles, where there is no

blood supply, are relatively or absolutely impermeable, to foreign proteins present in the blood. This was shown in the following series of experiments.

ENTRANCE OF EGG ALBUMEN INTO TUBERCLES.

In order to determine the entrance of proteins into tubercles, advantage was taken of the delicate and accurate method offered by the anaphylaxis reaction for the detection of small quantities of foreign proteins. The following experiments were performed:

Experiment 1.—A large guinea-pig, which had been inoculated subcutaneously with 0.01 mg. of human tubercle bacilli three months previously, was bled to the amount of 4 c.c., and an equal amount of fresh filtered four per cent solution of Merck's dried egg albumen powder was injected into the blood by the intracardiac route. After three hours the animal was bled to death and the blood defibrinated. Autopsy showed large caseous cervical glands and smaller tuberculous mediastinal and inguinal glands. The spleen was studded with tubercles, many of which were two to four millimeters in diameter. The liver and lungs showed a few tubercles one to two millimeters in diameter. Samples were taken aseptically from various tissues, ground up with quartz sand, emulsionized under aseptic precautions in 10 c.c. of water for each gram of substance. After filtering, the extract was injected intraperitoneally in various sized doses into 22 guinea-pigs. Most of these pigs died in four to six days with a peculiar gelatinous exudative peritonitis, great numbers of cocci being present in this exudate. Eighteen days after injecting the tissue extract, the survivors received by the peritoneal route an injection of 0.05 gm. Merck's egg albumen, to discover whether the tissue extracts had contained sufficient egg albumen to sensitize the injected pigs. The results were as follows:

	Sensitizing Dose	Result of Second Injection
1.	5 c.c. blood (undiluted)	Died in 18 minutes
2.	1 c.c. " "	" " 15 "
3.	0.1 c.c. " "	" " 20 "
4.	0.5 c.c. urine (undiluted)	" " 45 "
5.	4 c.c. spleen extract	Severe symptoms, temperature fell to 100°
6.	1 c.c. " "	" " " " 96°
7.	1 c.c. extract of caseous glands	Slight " " " " from 104° to 101°
8.	0.75 c.c. " " tubercles from liver	No definite symptoms. No fall in tem- perature
9.	0.50 c.c. " " " " "	No definite symptoms. Temperature 101°

On account of the large proportion of deaths from infection, the results of this series were not altogether satisfactory, but such experiments as could be completed indicated that there was less

sensitization by extracts of tuberculous liver tissue and caseous glands than by extracts of spleen tissue taken from between the tubercles.

Experiment 2.—In the second experiment the danger of peritonitis was avoided by making the sensitizing injections subcutaneously. A 400 gm. guinea-pig which had large caseous inguinal glands from injection of human tubercle bacilli, was given an injection of three cubic centimeters of a four per cent solution of egg albumen powder in the carotid artery. This animal was very sick when injected, and it was so nearly dead three hours later when bled to death that it could be but partly bled, only five to six cubic centimeters escaping; therefore, all the organs were left containing much blood, including the caseous lymph glands, the necrotic content of which, when removed by scraping, was somewhat blood tinged. The tissues and blood were extracted with 10 parts of water as before, and the extract was injected in doses of from one to five cubic centimeters into 19 guinea-pigs. In this experiment, the sensitizing dose chosen was evidently too large, since 18 of the animals reacted fatally to egg albumen 18 days later. It can only be stated that the animals sensitized with extracts of the caseous material did not die as quickly as the others, and the sole survivor was in this group. Presumably, the amount of blood present in the tissues was sufficient to produce a fatal sensitization in the doses used.

Experiment 3.—The difficulties disclosed in the two previous experiments were avoided in a third trial. Here a 400 gm. pig with a large mass of fluctuating tuberculous lymph glands received two cubic centimeters of a four per cent solution of Merck's egg albumen in the jugular vein. Three hours later it was bled to death, but bled poorly and much blood was left in the body. The liver was found riddled with small tubercles, the spleen was greatly enlarged and contained some good-sized necrotic areas. The inguinal glands contained a great amount of soft caseous material, part of which was removed in two separate portions without pressure and without appreciable contamination with blood. Specimens of blood, liver tissue, spleen tissue between the large tubercles, the two separate lots of caseous material, and the uncaseated peripheral gland substance itself were each ground with quartz sand, extracted six hours with repeated stirring in 10 volumes of sterile water, filtered, and the filtrate injected subcutaneously into guinea-pigs. After 18 days, each pig was injected intraperitoneally with .050 gm. Merck's egg albumen, with the results shown in the table on p. 371.

From these experiments, it seems evident that the egg albumen present in the circulating blood does not enter the caseous material which is shut off from the blood by proliferating tissue, during three hours after its intravascular injection, at which time the blood contains sufficient egg albumen to sensitize a guinea-pig when injected in a dose of 0.001 c.c.¹ It is not possible to tell whether

¹ It may be mentioned that our experiments differ radically in one result from those of Vaughan, Cumming, and McGlumphy (*Ztschr. f. Immunitätsf.* 1911, 9, p. 16), for they state that egg-white injected into the blood of rabbits disappears in one hour, although it may be found in the various organs after that time. We found that egg albumen injected into the blood of guinea-pigs remains in the blood at least three hours, when 0.001 c.c. of blood contains a sensitizing dose. How much longer than three hours the albumen remains in the blood, and how much smaller doses than 0.001 c.c. are capable of sensitizing, we did not determine.

the positive results obtained with the liver, spleen, and tuberculous glands depend on the egg albumen contained within the cells or that present in the blood in these tissues. We were unable to secure a sufficient number of guinea-pigs to investigate this point.

1. Blood		1 c.c.	Died in 20 minutes
2. "		0.1 c.c.	Severe reaction
3. "		0.02 c.c.	" "
4. "		0.01 c.c.	" "
5. Liver		2.0 c.c.	Moderately severe reaction
6. "		0.5 c.c.	Died after 2 hours
7. "		0.05 c.c.	" " " "
8. Spleen		1 c.c.	Moderate reaction
9. "		0.1 c.c.	Slight "
10. "		0.01 c.c.	" "
11. Gland tissue		1 c.c.	Died in 30 minutes
12. " "		0.1 c.c.	Moderate reaction
13. " "		0.01 c.c.	" "
14. Caseous material	Sample A.	2.0 c.c.	No reaction
15. " "		" " 0.5 c.c.	Slight or doubtful reaction
16. " "		" " 0.1 c.c.	Doubtful reaction
17. " "		" " 0.01 c.c.	No reaction
18. " "	Sample B.	2.0 c.c.	Doubtful reaction
19. " "		" " 0.5 c.c.	No reaction
20. " "		" " 0.1 c.c.	" "
21. " "		" " 0.01 c.c.	" "

SUMMARY.

A systematic consideration of the chemotherapy of tuberculosis rests on an investigation of the permeability of both the tubercle bacillus and the tuberculous lesion for chemical substances of different characters. It is shown that compounds of iodin injected into tuberculous animals enter glandular tubercles with readiness, so that the proportion of iodin in such tubercles is usually greater than it is in most other tissues except the kidney; furthermore it is greater in the caseous contents than in the cellular peripheries of the tubercles. Tuberculous eyes usually contain much more iodin than their normal mates. This property is shown not to depend on any specific character of the tubercle itself, for other

necrotic tissues also take up more iodin than normal tissues. The explanation offered is that normal cells are not perfectly permeable to iodids (except perhaps kidney cells) and lose this impermeability or semi-permeability when killed or injured, thus becoming entirely permeable for crystalloids present in the surrounding fluids. As the iodin content of the blood increases and decreases with absorption and elimination, so the iodin in the necrotic area, whether tuberculous or otherwise, varies, indicating an absence of any chemical or physical binding of the iodin in such areas. A simple, inert, colloid agar, implanted in the tissues, behaves in quite the same way.

Egg albumen injected into tuberculous pigs is found, by means of the anaphylaxis reaction, to penetrate the avascular tubercles but little if at all, even when present in the blood in large amounts. This agrees with the hypothesis that the passage of iodin from the blood into the tubercles is a purely physical matter, the crystalloidal iodin compounds diffusing through the inert colloidal solution of a necrotic area practically unimpeded, while the colloidal egg albumen, according to the law of colloidal diffusion, is practically unable to diffuse through such a colloidal solution.

No evidence could be found of any tendency for iodin compounds of whatever nature to accumulate in tubercles or other necrotic areas, or to persist in such areas when disappearing from the normal tissues and the blood.

Exudates contain approximately the same proportion of iodin as the blood of the same animals, and hence any area with inflammatory edema and congestion will commonly show more iodin than normal tissues, although not usually more than the blood. No evidence was found of any specific entrance or fixation of iodin in inflammatory exudates. The iodin is distributed about alike in the fluid and solid portions of the exudate, indicating simple diffusion. Of normal tissues only the kidney seems to contain approximately as much iodin as the blood of the same animal. This may have some bearing upon its excretory function, since it indicates a greater permeability of renal cells than of other gland cells for iodids.